

WE CLAIM:

1. A single-chain multimeric polypeptide having G-CSF activity, comprising at least two monomeric units independently selected from (a) hG-CSF with the amino acid sequence shown in SEQ ID NO:1 and (b) variants of hG-CSF, said monomeric units being linked via a peptide bond or a peptide linker, the polypeptide comprising at least one covalently bound polymer molecule selected from the group consisting of linear and branched polyalkylene oxides.
2. The single-chain multimeric polypeptide of claim 1, wherein the polymer molecule is polyethylene glycol.
3. The single-chain multimeric polypeptide of claim 2, wherein at least one polyethylene glycol molecule is bound to a lysine residue.
4. The single-chain multimeric polypeptide of claim 1, wherein at least one of said monomeric units has the amino acid sequence of hG-CSF (SEQ ID NO:1).
5. The single-chain multimeric polypeptide of claim 4, comprising two monomeric units, wherein both of said monomeric units have the amino acid sequence of hG-CSF (SEQ ID NO:1).
6. The single-chain multimeric polypeptide of claim 1, wherein at least one of said monomeric units is a variant of hG-CSF comprising at least one substitution, addition or deletion compared to SEQ ID NO:1.
7. The single-chain multimeric polypeptide of claim 6, wherein at least one of said monomeric units is a variant of hG-CSF comprising at least one amino acid residue modification selected from the group consisting of: introduction of a lysine, cysteine, aspartic acid, glutamic acid or histidine residue; and removal of a lysine, cysteine, aspartic acid, glutamic acid or histidine residue.

8. The single-chain multimeric polypeptide of claim 6, comprising two monomeric units, wherein both of said monomeric units have at least one amino acid residue modification compared to hG-CSF.
- 5 9. The single-chain multimeric polypeptide of claim 1, comprising two monomeric units, wherein one of said monomeric units has the amino acid sequence of hG-CSF (SEQ ID NO:1) and the other monomeric unit is a variant of hG-CSF
- 10 10. A single-chain multimeric polypeptide having G-CSF activity, comprising at least two monomeric units independently selected from (a) hG-CSF with the amino acid sequence shown in SEQ ID NO:1 and (b) variants of hG-CSF, said monomeric units being linked via a peptide bond or a peptide linker, wherein the polypeptide has at least one non-polypeptide moiety covalently bound to an attachment group of the polypeptide and exhibits an *in vitro* bioactivity in the range of about 2-30% of the bioactivity of non-conjugated hG-CSF as determined by the luciferase assay described herein.
- 15 11. The single-chain multimeric polypeptide of claim 10, comprising two monomeric units.
12. The single-chain multimeric polypeptide of claim 11, wherein each of said two monomeric units comprises the amino acid sequence of hG-CSF as shown in SEQ ID NO:1.
- 20 13. The single-chain multimeric polypeptide of claim 10, wherein at least one monomeric unit is a variant of hG-CSF with at least one amino acid residue modification selected from the group consisting of: introduction of a lysine, cysteine, aspartic acid, glutamic acid or histidine residue; removal of a lysine, cysteine, aspartic acid, glutamic acid or histidine residue; introduction of an N- or O-glycosylation site; and removal of an N- or O-glycosylation site.
- 25 14. The single-chain multimeric polypeptide of claim 10, wherein at least one monomeric unit differs from the corresponding wild-type polypeptide in that least one attachment site for a non-polypeptide moiety has been introduced in a position that in the wild-type polypeptide is occupied by a surface-exposed amino acid residue, and/or wherein at least
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one amino acid residue that is surface-exposed in the wild-type polypeptide has been removed.

15. The single-chain multimeric polypeptide of claim 10, comprising at least one covalently
 5 bound non-polypeptide moiety selected from the group consisting of polymer molecules, lipophilic compounds, oligosaccharide moieties and organic derivatizing agents.

16. The single-chain multimeric polypeptide of claim 15, comprising at least one covalently
 10 bound polymer molecule selected from the group consisting of linear and branched polyalkylene oxides.

17. The single-chain multimeric polypeptide of claim 16, wherein the polymer molecule is polyethylene glycol.

18. The single-chain multimeric polypeptide of claim 11, wherein at least one of said two
 15 monomers differs from wild-type human G-CSF in that at least one lysine residue has been removed.

19. The single-chain multimeric polypeptide of claim 11, wherein at least one of said two
 20 monomers differs from wild-type human G-CSF in that at least one lysine residue has been introduced.

20. The single-chain multimeric polypeptide conjugate of claim 10 having an *in vitro* bioactivity in the range of about 3-25% of the bioactivity of non-conjugated hG-CSF as determined by the luciferase assay described herein.
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21. The single-chain multimeric polypeptide conjugate of claim 10 having an *in vitro* bioactivity in the range of about 4-20% of the bioactivity of non-conjugated hG-CSF as determined by the luciferase assay described herein.
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22. A nucleotide sequence encoding a single-chain polypeptide according to claim 1.

23. An expression vector comprising a nucleotide sequence according to claim 22.

24. A recombinant host cell comprising an expression vector according to claim 23.

25. A method for preparing a single-chain multimeric polypeptide having G-CSF activity,
5 comprising culturing a recombinant host cell according to claim 24 comprising a single
nucleotide sequence encoding said polypeptide in a suitable culture medium under con-
ditions permitting expression of the nucleotide sequence, and recovering the resulting
polypeptide from the cell culture

10 26. The method of claim 25, further comprising subjecting the polypeptide to conjugation *in*
vitro with a non-polypeptide moiety under suitable reaction conditions to result in a
polypeptide conjugate.

27. A composition comprising a single-chain multimeric polypeptide according to claim 1
15 together with at least one excipient or vehicle.

28. A method of treating a mammal having a general haematopoietic disorder, including
disorders arising from chemotherapy, radiation therapy or bone marrow transplantations,
treatment of AIDS or other immunodeficiency diseases, treatment of leukopenia and
20 treatment of acute myeloid leukaemia, comprising administering to a mammal in need
thereof an effective amount of a single-chain multimeric polypeptide according to claim
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29. A single-chain multimeric polypeptide having G-CSF activity, comprising at least two
25 monomeric units independently selected from (a) hG-CSF with the amino acid sequence
shown in SEQ ID NO:1 and (b) variants of hG-CSF, said monomeric units being linked
via a peptide bond or a peptide linker, wherein the polypeptide has at least one non-
polypeptide moiety covalently bound to an attachment group of the polypeptide.

30. The single-chain multimeric polypeptide of claim 29, comprising at least one covalently
30 bound non-polypeptide moiety selected from the group consisting of polymer molecules,
lipophilic compounds, oligosaccharide moieties and organic derivatizing agents.

31. A single-chain multimeric polypeptide having G-CSF activity, comprising at least two monomeric units independently selected from (a) hG-CSF with the amino acid sequence shown in SEQ ID NO:1 and (b) variants of hG-CSF, said monomeric units being linked via a peptide bond or a peptide linker, the polypeptide comprising at least one covalently bound polyethylene glycol molecule.

32. The single-chain multimeric polypeptide of claim 31, comprising at least one polyethylene glycol molecule bound to a lysine residue.

33. The single-chain multimeric polypeptide of claim 31, comprising two monomeric units comprising the amino acid sequence of hG-CSF as shown in SEQ ID NO:1.

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